Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

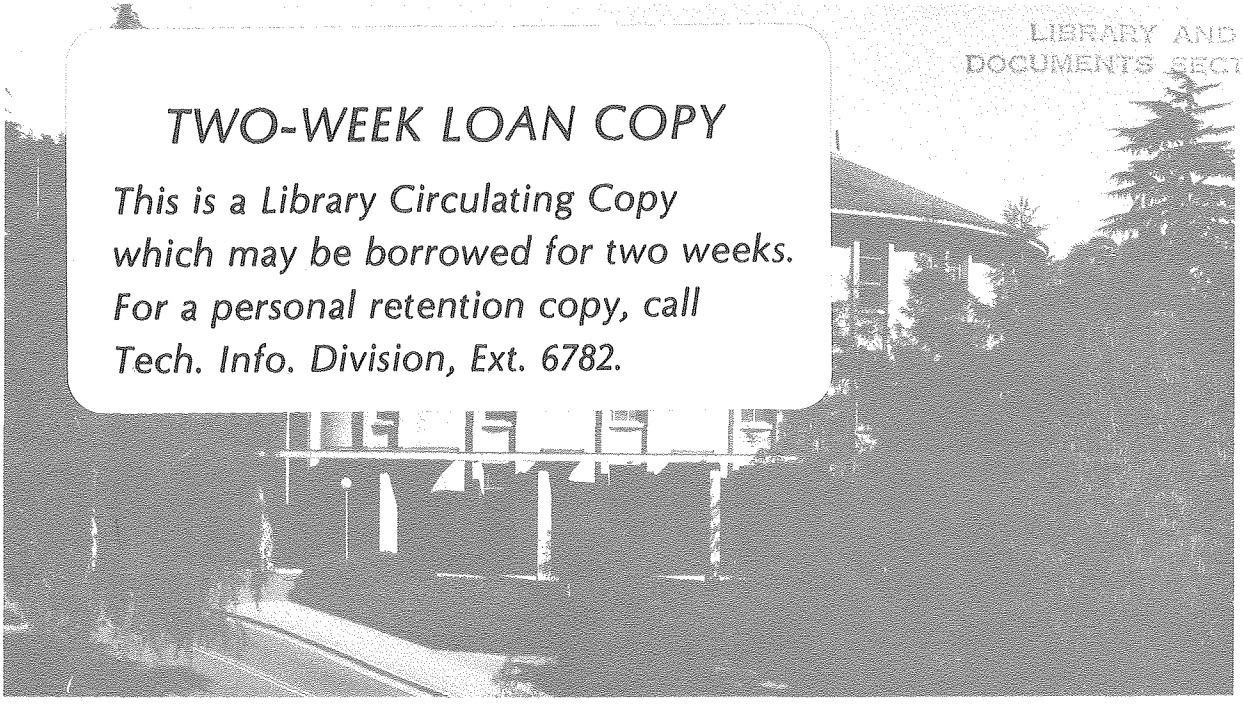
CHEMICAL BIODYNAMICS DIVISION

Presented at the Psychophysiological Symposia of the XXII International Congress of Psychology, Leipzig, E. Germany, July 6-11, 1980

TESTS OF THE PROTEIN-SYNTHESIS HYPOTHESIS OF FORMATION OF LONG-TERM MEMORY

Mark R. Rosenzweig, Edward L. Bennett and James F. Flood

September 1980



Prepared for the U.S. Department of Energy under Contract W-7405-ENG-48

DISCLAIMER

TESTS OF THE PROTEIN-SYNTHESIS HYPOTHESIS OF FORMATION OF LONG-TERM MEMORY

Mark R. ROSENZWEIG[a], Edward L. BENNETT[b] and James F. FLOOD[c]

[a]Department of Psychology and [b]Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720 U.S.A.

[c]Department of Psychology, University of California, Los Angeles, California 90024 U.S.A.

Running head: Protein synthesis and formation of long-term memory

or

Protein synthesis and long-term memory

This manuscript was printed from originals provided by the author.

TESTS OF THE PROTEIN-SYNTHESIS HYPOTHESIS OF FORMATION OF LONG-TERM MEMORY

Mark R. ROSENZWEIG[a], Edward L. BENNETT[b] and James F. FLOOD[c]

[a]Department of Psychology and [b]Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720 U.S.A.

[c]Department of Psychology, University of California, Los Angeles, California 90024 U.S.A.

Summary

The hypothesis that synthesis of protein in the nervous system is required for formation of long-term memory is supported by many studies, but apparent limitations have been suggested and competing interpretations have been offered. Several alternative hypotheses have also been proposed and data have been offered in support of them. Thus, it was claimed that inhibition of protein synthesis could overcome effects of weak training but not of strong training. We found, however, that amnesia could be caused even after rather strong training if the inhibition was maintained at a high level for a long enough period after training. It has been proposed that protein synthesis inhibitors (PSIs) are effective against memory because they interfere with catecholamine systems. We have found, however, that PSI doses that produce amnesia have only small effects on catecholamine synthesis and that catecholamine inhibitors are not equivalent, in several respects, to PSIs as amnestic agents. The possibilities that PSIs cause amnesia by producing some non-specific side effect such as sickness or by depleting brain proteins or by increasing the level of some metabolite have

been rendered unlikely by many control experiments. Recently it has been claimed that earlier administration of a PSI can actually protect against amnesic effects of the PSI given close to the time of training and that the degree of amnesia is not related to the level of inhibition of protein synthesis at the time of training. In extensive experimentation we found that the only weak indication of a protective effect occurred for only a single drug-task combination and that under most conditions the degree of amnesia was predicted by the level of inhibition. One role of proteins in memory storage may be in formation of new or modified synaptic connections. This would require transport of the proteins from the cell body out along axons and/or dendrites. Agents that inhibit axonal transport have recently been found to produce amnesia if they are injected close to the time of training. Thus it appears that the role of proteins in memory storage can be affected not only by inhibiting their synthesis but also by preventing their transport to synaptic sites.

Introduction

Within the overall attempt to find the basic processes and sites of plasticity in the nervous system, a major hypothesis has been that synthesis of protein is required for formation of long-term memory. Results of many studies conducted during the past decade have supported this hypothesis, but different limitations have been suggested and competing interpretations have been offered. Several alternative hypotheses have also been proposed and data have been offered in favor

of them. We will review some of the results both for and against the protein-synthesis hypothesis.

The basic findings are these: If rodents or chicks are given an inhibitor of protein synthesis shortly before a brief period of training, this does not affect learning or short-term memory, but a sufficient level and duration of inhibition prevents formation of long-term memory or causes the memory trace to be weak. In the chick given either anisomycin (ANI) or cycloheximide (CYCLO) within a period of 30 min. pretraining to 10 min. posttraining, memory is normal for about 30 min. but is substantially impaired at 60 min. or later (GIBBS and NG, 1977). Results obtained with a variety of agents suggest that there are two or more successive stages in the formation of memory, with only the long-term stage requiring synthesis of proteins.

Producing amnesia after strong training

One reason for doubting the protein-synthesis hypothesis was the report that in mice inhibition of protein synthesis could overcome the effects of weak training but could not cause amnesia after strong training. We found, however, that amnesia could be caused even after rather strong training if the inhibition of protein synthesis was maintained at a high level for a long enough period after training. (See Figure 1.) The stronger the training, the longer inhibition must be maintained in

Figure 1 around here

order to cause amnesia. This was found for both step-through passive avoidance training (FLOOD et al., 1973) and active avoidance training in

a T-maze (FLOOD et al., 1975).

Interference with catecholamine systems?

A hypothesis that has received considerable attention since 1972 is that protein synthesis inhibitors (PSIs) are effective against memory because they interfere with catecholamine systems, as shown in Figure 2. We have found that while PSIs do inhibit synthesis of catecholamines, doses of ANI that are effective in producing amnesia cause only about

Figure 2 around here

15 to 20% reduction in catecholamine synthesis--far less than the 50-85% claimed in some reports. We have also found that catecholamine inhibitors (CAIs) are not equivalent, in several respects, to PSIs as amnestic agents, as the following types of evidence demonstrate:

(a) When passive avoidance training was weak, a pretrial injection of either a CAI or a PSI caused amnesia (Figure 2). Weak shock caused weak learning. On the other hand, when training was strong,

Figure 3 around here

a series of three successive injections of PSI (one injection pre-training and two posttraining) caused amnesia, whereas a similar series of injections of a CAI did not cause amnesia (Figure 4) (BENNETT, ROSENZWEIG and FLOOD, 1979).

Figure 4 around here

(b) Under these training conditions, substituting one injection of CYCLO for one of the three injections of ANI also caused amnesia, but substituting a CAI for one of the injections of ANI did not cause amnesia (Figure 5)(BENNETT, ROSENZWEIG and FLOOD, 1979).

Figure 5 around here

(c) Strong evidence differentiating CAIs from PSIs is provided by recent experiments on effects of localized injections of drugs into various brain sites (FLOOD, SMITH and JARVIK, 1980). Main results are shown in Table 1. In the brainstem, where both norepinephrine

Table 1 around here

and dopamine are synthesized, CAIs caused amnesia but PSIs did not. In the amygdala, which receives both noradrenergic and dopaminergic fibers, both PSIs caused amnesia; so did DDC (which inhibits conversion of dopamine to norepinephrine), but AMPT (which interferes with catecholamine synthesis) did not cause amnesia at this site. In the caudate-putamen which receives a major projection of dopaminergic fibers, AMPT was an effective amnestic agent whereas DDC was not; both PSIs also caused amnesia when injected here. It thus appears that there are rather specific effects of CAIs and PSIs on memory and that the actions of PSIs cannot be attributed to their effects on catecholamine levels. The small amounts of agents required for effective brain injections minimizes the likelihood of side effects

that might interfere with interpretation of these experiments.

Problems of non-specific side effects.

Do PSIs cause amnesia by producing some non-specific side effect such as sickness or by depleting brain proteins or increasing the level of some metabolite? These problems have been considered extensively by SQUIRE (e.g., 1976), among others. One test of such alternatives has been to administer the PSI an hour or more before or after training instead of very close to the time of training. Non-specific effects should be equally severe in both cases, but only the administration of the PSI close to training has been found to cause amnesia. The possibility of subtle side effects at the time of training was tested by giving different groups of mice either a large dose of ANI 5 hours before training or a low dose 20 min. before training (DAVIS, et al., 1980). At the time of training, the large dose reduced locomotor activity and caused overt symptoms of sickness whereas animals with the low dose appeared like saline controls, but the low recent dose caused greater inhibition of protein synthesis as shown in Figure 6 at time T and thereafter. Degree of amnesia

Figure 6 around here

was found to be related to the level of inhibition of protein synthesis in the immediate posttraining period rather than to the side effects.

Does inhibition affect retrieval rather than storage?

It has been claimed that inhibition of protein synthesis affects retrieval rather than storage of information and that the effect on memory is transitory; that is, it has been reported in some cases that

subjects show amnesia if tested one day after training but remember if tested at later times. We have not seen reports of recovery if amnesia was shown later than 24 hours posttraining. At 24 hours post-training, apparent amnesia may reflect other effects, so memory should regularly be tested at later intervals. Non-contingent "reminder" procedures have been reported to aid recovery of memory. We have found that testing for memory can be an effective reminder procedure if there is a weak memory. If an initial test of passive avoidance shows an

Figure 7 around here

intermediate latency, this indicates the presence of some memory, and the latency then usually becomes longer on successive days of testing, indicating strengthening of memory (Figure 7). But when initial tests yield very short latencies, indicating no memory of the shock training, then the latencies remain short throughout the four days of testing, indicating no recovery (DAVIS et al., 1978). Thus, sufficient inhibition of protein synthesis can prevent storage of memory, and in this case no retrieval occurs even when reminders are given.

Is there a protective effect of inhibition?

A recent challenge to the protein synthesis hypothesis claims that the degree of amnesia is not related to the level of inhibition of protein synthesis at the time of training and that an earlier administration of CYCLO can actually protect against amnesic effects of CYCLO given close to the time of training (RAINBOW, HOFFMAN and FLEXNER, 1980). This alleged protective effect was inferred from the results of a single

experiment that used only 7-11 mice per condition; it involved single-trial passive avoidance training with memory tested 24 hours later. The results were claimed to cast doubt on the overall hypothesis that protein synthesis is required for long-term memory storage. We have attempted to replicate these results and to explore the possibility of a protective effect in a larger experimental design (Table 2) that included tests at 7 days

Table 2 around here

as well as 1 day, active as well as passive avoidance training, and use of ANI as well as CYCLO (GROVE et al., in preparation). We obtained weak indications of a protective effect of an earlier injection of CYCLO, but these were not statistically significant even with Ns of 25 per condition. Moreover, these indications were found only for passive avoidance training; with active avoidance, the reverse of a protective effect was found--the double injection was more effective than a single one. Also, the use of ANI showed the reverse of a protective effect for passive avoidance. Thus the only weak indication of a protective effect occurred for only a single drug-task combination; under most conditions, the degree of amnesia was predicted by the level of inhibition of protein synthesis.

Axonal transport and long-term memory

Memory-related protein(s) synthesized in the posttraining period may play several different roles in memory storage. Some of the memory-specific proteins may play purely intracellular functions. One role which we favor is that structural proteins and receptor proteins may participate in forma-

tion of new or modified synaptic connections. This would require transport of the proteins from the cell body out along axons and/or dendrites. Colchicine and vinblastine, agents that inhibit axonal transport, have recently been found to produce amnesia if they are injected close to the time of training (Figure 8) (FLOOD, LANDRY, BENNETT & JARVIK, in preparation). Thus it appears that the role of protein(s) in memory storage can be affected not only by inhibiting their synthesis but also by preventing their transport to synaptic sites.

In conclusion, the hypothesis that protein synthesis is required for long-term memory appears both to have survived many challenges and to continue to stimulate informative research.

Acknowledgments

The research of the authors received support from ADAMHA Grant R01MH26704, NIMH Grant 26608, and from the Division of Biomedical and Environmental Research of the U.S. Department of Energy under contract W-7405-ENG-48.

References

BENNETT, E.L., M.R. ROSENZWEIG & J.F. FLOOD: Role of neurotransmitters and protein synthesis in short-and long-term memory. In J. OBIOLS et al. (eds.): Biological Psychiatry Today. Elsevier/North Holland Biomedical Press, Amsterdam, 1979. Pp. 211-219.

DAVIS, H.P., M.R. ROSENZWEIG, E.L. BENNETT & A.E. ORME: Recovery as a function of the degree of amnesia due to protein synthesis inhibition. Pharmacol. Biochem. Behavior 8, 701-710 (1978).

DAVIS, H.P., M.R. ROSENZWEIG, E.L. BENNETT & L.R. SQUIRE: Inhibition of cerebral protein synthesis: Dissociation of nonspecific effects and annesic effects. Behavioral & Neural Biol. 28, 99-104 (1980).

FLOOD, J.F., E.L. BENNETT, D.W. LANDRY & M.E. JARVIK: Dependency of long-term memory on protein synthesis and its transport. (Manuscript submitted)

FLOOD, J.F., E.L. BENNETT, A.E. ORME & M.R. ROSENZWEIG; Effects of protein synthesis inhibition on memory for active avoidance training. Physiol. & Behavior, 14, 177-184 (1975).

FLOOD, J.F., E.L. BENNETT, M.R. ROSENZWEIG & A.E. ORME: The influence of duration of protein synthesis inhibition on memory. Physiol. & Behavior, 10, 555-562 (1973).

FLOOD, J.F., G.E. SMITH & M.E. JARVIK: A comparison of the effects of localized brain administration of catecholamine and protein synthesis inhibitors on memory processing. Brain Res., in press (1980).

GIBBS, M.E. & K.T. NG: Psychobiology of memory: Towards a model of memory formation. Biobehav. Rev. 1, 113-136 (1977).

GROVE, E., H.P. DAVIS, M.R. ROSENZWEIG & E.L. BENNETT: Investigation of the reported protective effect of cycloheximide on memory. (In preparation).

RAINBOW, T.C., P.L. HOFFMAN,& L.B. FLEXNER: Studies of memory: A reevaluation in mice of the effects of inhibitors on the rate of synthesis of cerebral proteins as related to amnesia. Pharmacol. Biochem. Behav. 12, 79-84 (1980).

SQUIRE, L.R. & S.H. BARONDES: Amnesic effect of cycloheximide not due to depletion of a constitutive brain protein with short half-life. Brain Research, 103, 183-190 (1976).

Key Words

Amnesia

Anisomycin

Axonal transport and memory

Catecholamines and memory

Colchicine

Long-term memory formation

Memory formation

Protein synthesis inhibition

Synaptic connections and memory

Figure Captions

Figure 1. Percentages of animals showning amnesia on retest after having received one of four levels of passive avoidance training and one, two, or three successive injections of anisomycin (ANI, 0.5 mg/mouse) at 2-hr. intervals. Of 96 saline control mice, only 2 showed amnesia; these control data are not included in the graph. All drug groups showing at least 25% amnesia differed at the 0.05 level or better from controls that received the same training (Fisher Exact Probability test). The levels of training were achieved by selecting animals that showed the following values of training and escape latencies, respectively: I. 1-4.9 sec., 0.01-0.04 min. II. 5-8.4 sec., 0.01-0.04 min. III. 1-4.9 sec., 0.05-0.08 min. IV. 5-8.4 sec., 0.05-0.08 min. (From FLOOD et al., 1973).

Figure 2. Effects of the catecholamine inhibitors diethyldithiocarbamate (DDC), tetrabenazine (TB), and α-methyl paratyrosine (AMPT) alone and in combination with anisomycin (ANI) on cerebral concentration of tyrosine (TYR), dopamine (DA), and norepinephrine (NE). The CAIs were administered 1 hr. after the ANI and the mice were sacrificed 1 hr. later. Drugs were administered by subcutaneous injection. (From BENNETT et al., 1979).

Figure 3. Comparison of the amnesic effect of inhibitors of catecholamine and protein synthesis as a function of shock intensity. The drug dosages are shown in Fig. 2. At the lowest footshock intensity used for training, both the CAIs and ANI produced amnesia. As the footshock intensity increased, the effectiveness of the CAIs and a single injection of ANI as amnestic

agents decreased in parallel, and at the highest intensity, none was effective. Therefore, this test design did not provide evidence that the effective mode of action of these drugs differed. A difference was shown, however, under the conditions of Fig. 4.

Figure 4. This experiment compared the effect of three successive injections of ANI or the catecholamine inhibitors on retention of a passive avoidance task. Drug dosages as shown in Figure 2. When tested one week later, ANI-injected mice were amnesic, but the mice administered catecholamine inhibitors were not. (N = 20/group). (from BENNETT et al., 1979).

Figure 5. With the one-trial passive avoidance task, no amnesia was obtained when a catecholamine inhibitor was substituted for the second of a series of ANI injections. (Drug dosages are shown in Figure 6.) However, amnesia was obtained when cycloheximide, (100mg/kg) another protein synthesis inhibitor, was substituted for anisomycin. (N =20/group). (From BENNETT et al., 1979).

Figure 6. Percentage inhibition of protein synthesis by ANI 210 mg/kg (○) and ANI 30 mg/kg (◇) is presented in relation to training time (T). Five mice were used for each data point, and the standard deviations are shown by the vertical bars. These inhibition curves have been derived, in part, from numerous other experiments carried out in this laboratory. (From DAVIS et al., 1980).

Figure 7. Median step-through latencies (STL) for mice categorized solely on the basis of their initial STL, irrespective of drug or training-test interval. ●———● STL 1-7 sec., including the following animals: Saline, N = 27; Ani 1 mg, N = 12; Ani 7 mg or Ani 1 mg x 7, N =73; Total N = 112. o———o STL 8-200 sec.: Saline, N = 52; Ani 1 mg, N = 43; Ani 7 mg or Ani 1 x7, N = 81; Total N = 176. ▫———▫ STL 201-600 sec: Saline, N = 100; Ani 1 mg, N = 26; Ani 7 mg or Ani 1 mg x 7, N = 10; Total N = 136. (DAVIS, et al., 1978).

Figure 8. Mice were trained on a T-maze after pretraining administration of Ani (20 mg/kg). Immediately following training, colchicine (60mg/kg), vinblastine (6mg/kg), or saline was administered. Retention was determined one week after training. Under the conditions of training used in this experiment, only mice that received both ANI and an inhibitor of protein synthesis were amnesic.

Table 1

Effects of Brain Injections on Memory

(Percentages of subjects not remembering at test)

	Catecholamine Inhibitors		Protein-synthesis Inhibitors	
Site of injections	AMPT	DDC	ANI	CYCLO
Brainstem	75**	80**	27	26
Amygdala	27	82**	70*	79**
Caudate-Putamen	74*	35	83*	82**
Hippocampus	71**	80**	73**	89**
Septum	20	20	20	25

* $p < .01$ versus saline controls

** $p < .001$ versus saline controls

Ns ranged from 15 to 25 per drug group; each drug group had a saline control injection group with a similar N.

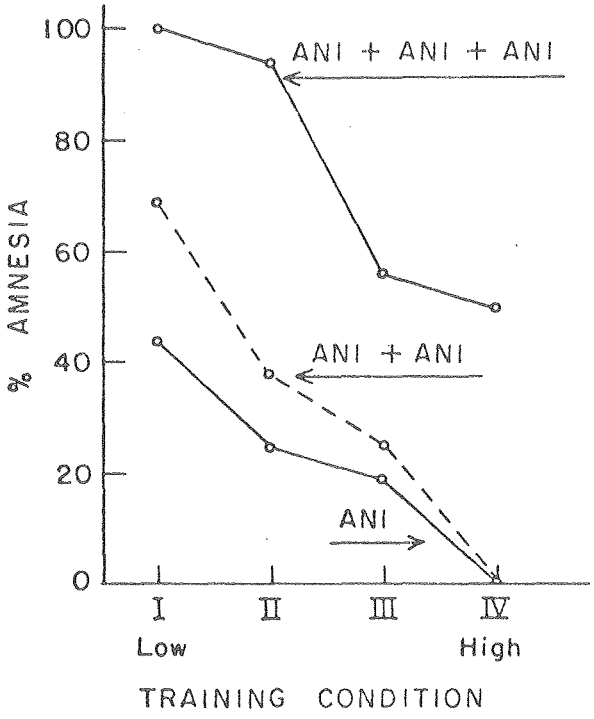
Table 2

Research Designs to Study Possible Protective Effect of Cycloheximide

(Entries show doses in mg/kg and one or two injections)

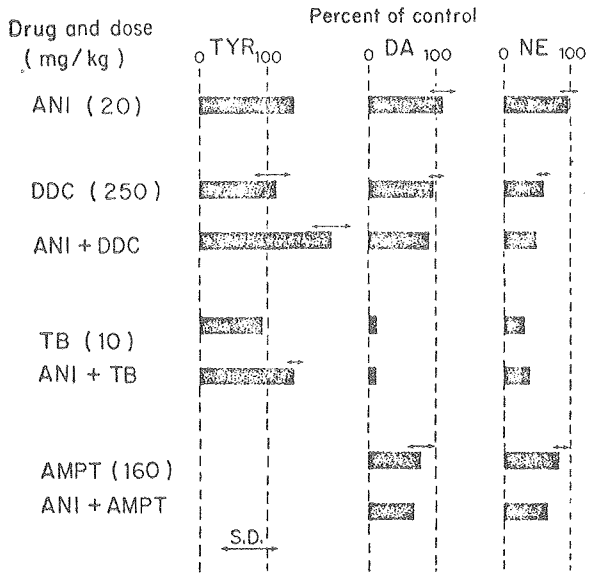
Cycloheximide	Anisomycin
← Passive Avoidance	← Passive Avoidance
Test at 1 day	Test at 1 day
30	30
120	120
120/30	120/30
Test at 7 days	Test at 7 days
30	30
120	120
120/30	120/30
← Active avoidance	
Test at 7 days	
30	
120	
120/30	

Each block included saline control groups as well as drug groups. The design of RAINBOW et al. (1980) employed only the conditions included within the upper left block, whereas our study included all the conditions shown.



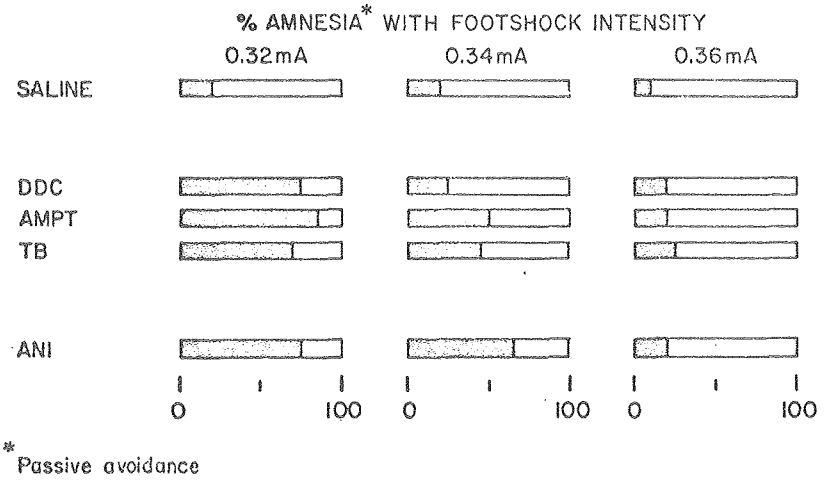
Rosenzweig et al.

Figure 1



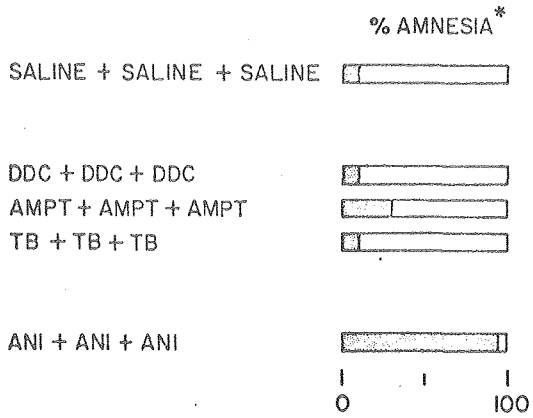
Rosenzweig et al.

Figure 2



Rosenzweig et al.

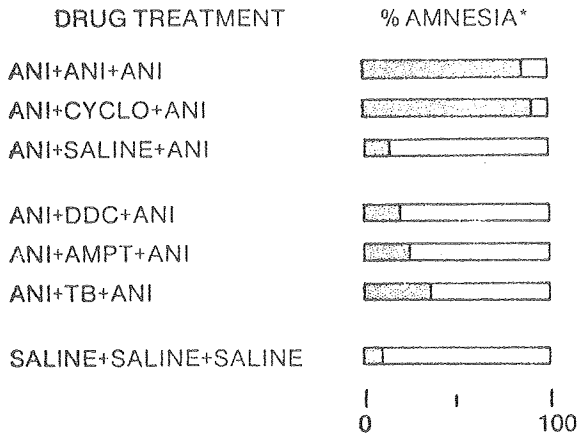
Figure 3



*Passive avoidance; footshock intensity, 0.36mA

Rosenzweig et al.

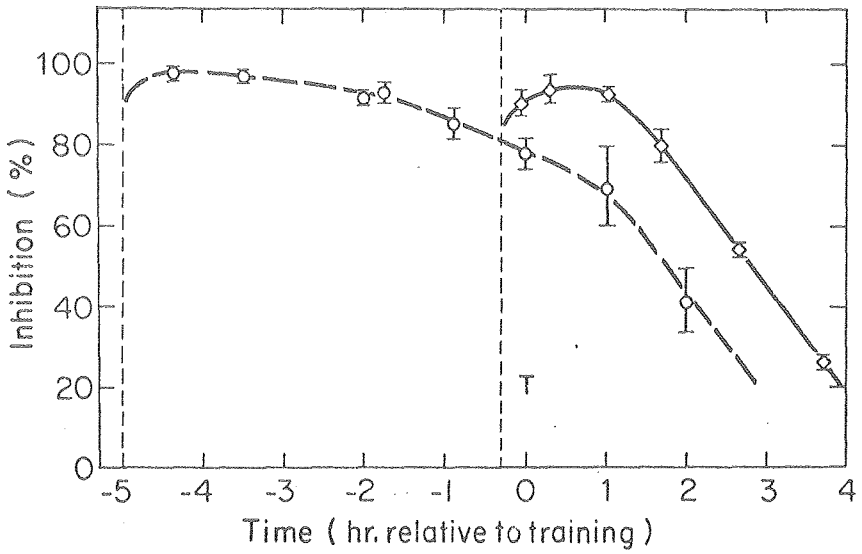
Figure 4



*Passive avoidance; footshock intensity, 0.36mA

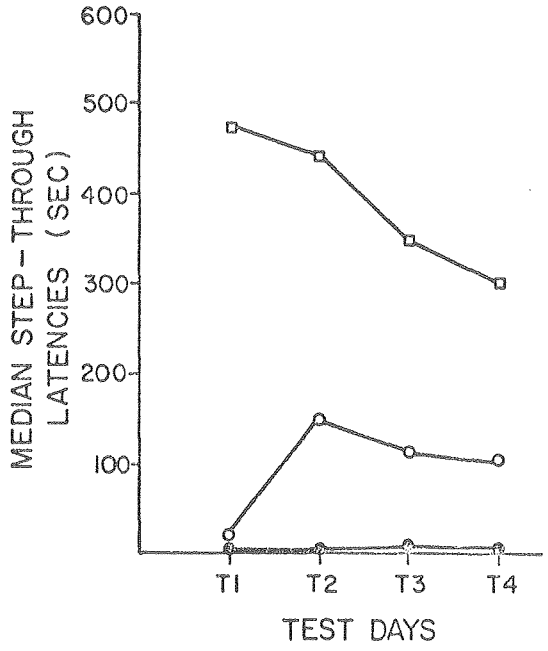
Rosenzweig et al.

Figure 5



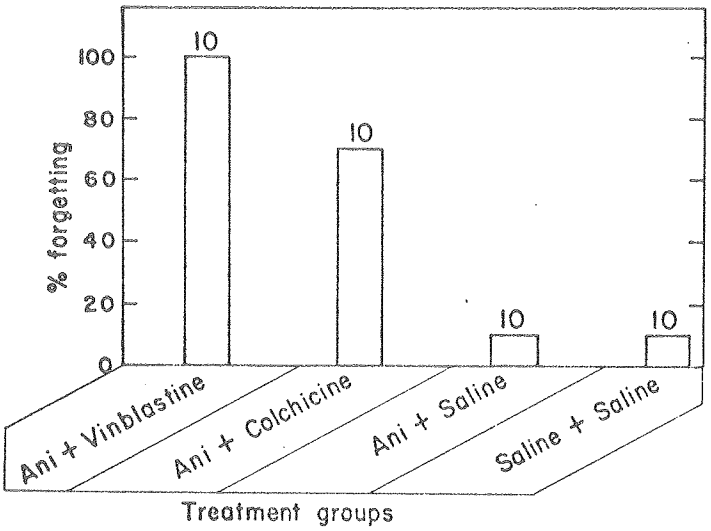
Rosenzweig et al.

Figure 6



Rosenzweig et al.

Figure 7



Rosenzweig et al.

Figure 8